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<b>(54) Title:</b> COMPOSITIONS FOR LUBRICATING AND SEPARATING TISSUES AND BIOLOGICAL MEMBRANES  <b>(57) Abstract</b>  The invention comprises a composition for lubricating and separating membranes from adjacent membranes or membranes from adjacent cells or tissues comprising a hydrophobized polymer which is a biologically acceptable water soluble cationic polymer with hydrophobic groups covalently bonded thereto and binds to a membrane. The membrane has on its surface a mesothelial, synovial, or mucus layer.		

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**COMPOSITIONS FOR LUBRICATING AND SEPARATING TISSUES AND  
BIOLOGICAL MEMBRANES**

5       The invention relates to a composition for lubricating and separating membranes from adjacent membranes or membranes from adjacent cells or tissues comprising a hydrophobized polymer. Preferably, the composition is used for preventing postsurgical adhesions, i.e. *de novo* adhe-  
10       sions which normally are caused by normal surgical manipulations and unintentional tissue injury.

      Vital tissues such as blood vessels or organs are coated with mucous, serous, synovial and endothelial membranes so that they can function independently of each  
15       other. The peritoneum, i.e. the serous membrane lining the abdominopelvic walls and investing the viscera as well as the pericardial and pleural sacs, has a smooth surface and forms a double-layered sac which is closed in the male and is continuous with the mucous membrane of the uterine tubes  
20       in the female. The peritoneal, pericardial and pleural membranes consist of a single layer of mesothelial cells, which is covered with a thin film of peritoneal fluid. The components of the membranes as well as the covering layer of fluid have several functions, e.g. the lubrication of  
25       the enclosed organs, unrestricted mobility being provided.

      The protective membrane is thus very thin and comprises a thin layer of connective tissue covered with a monolayer of mesothelial cells and only a few molecules thick layer of mainly phospholipids. When such a membrane  
30       is exposed to a physical, chemical or microbial challenge many potent substances harmful to the membrane are often released in response thereto. The structure and function of the membrane is consequently easily destroyed in connection with trauma, ischemia, and infection. After an irritation  
35       of the stress-sensitive membrane, e.g. only by the desiccation or abrasion of the membrane surfaces during

surgery, it will rapidly be covered with a fibrin clot. Since the plasminogen activating activity (i.e. the fibrinolytic capacity) is reduced after trauma, the fibrin clots will later on become organized as fibrous adhesions, i.e. small bands or structures by which adjacent serous or synovial membranes adhere in an abnormal way. Surgical operations, infection or inflammation in those parts of the body which are coated with serous or synovial membranes can result in adhesive inflammation regardless of the size of the affected area. The adhesions between vital tissues are formed within the first few days following surgery trauma or infection and may be observed not only in particular portions of the body but in all vital tissues. Such adhesions between for example intestines or intestines and the abdominal wall are the result of the often unnoticed tissue damage as desiccation and they occur for various reasons including mechanical and chemical stimulations of vital tissues accompanying surgical manipulations, postoperative bacterial infection, inflammation or further complications.

Adhesion of vital tissues, large or small, may be observed in most surgical fields and the postsurgical adhesions obtained are the result of a natural wound healing response of tissue damage occurring during surgery. In fact, it has been reported that of all patients undergoing abdominal surgery at one hospital over a four-year period, 93 % were found to have adhesions from previous operations. In 1994 surgeons in the US performed about 400,000 operations in which adhesions were removed. It is thus of great interest to produce measures for preventing such post-operative adhesions between vital tissues.

In summary, all tissues having contact with each other have a tendency to adhere to each other by fibrinous and fibrous adhesions. This is so for surfaces covered with mesothelium, i.e. peritoneum, pleura and pericardium, surfaces covered with synovia, i.e. joints and tendon sheets,

and for special surfaces such as the surfaces in the eye and the middle ear. Such adhesions also occur between different blood corpuscles and the inside of a blood vessel. The adhesions created by development of fibrin or connective tissue are unwanted since they result in a reduced function and a possible disease. In order to prevent this Nature has developed a system, whereby the surfaces are covered with layers of special components with the function of lubricating and separating tissues. These layers are, however, easily destroyed and this can have catastrophic consequences. While waiting after surgery for the body to produce new protective layers it is important to supply the corresponding protection from the outside in an effective way. Furthermore, it is important to prevent or reduce the infection and/or the inflammation obtained after surgery as well as the accompanying fibrin formation.

A number of methods for limiting the formation of surgical adhesion have been studied with some encouraging but often ambiguous results. However, most efforts made to avoid or reduce postoperative peritoneal adhesions have finally been abandoned. Among the methods used prevention of fibrin formation, reduction of fibrin formation, surface separation, and surgical techniques can be mentioned.

Numerous investigations have been carried out in which barriers are placed at a site of injury in order to prevent fibrin bridge formation between the injured tissue and neighboring organs. Such barriers include resorbable materials, such as enzymatically degradable oxidized regenerated cellulose, and slowly dissolving physiochemically crosslinked hydrogels of the Pluronic™ type.

Most methods of limiting postsurgical adhesion formation have also focused on providing wound separation by placing a material between the tissues. In addition, several types of viscous polymer solutions such as polyvinylpyrrolidone, sodium carboxymethyl cellulose, dextrans,

and hyaluronic acid have been added before and/or at the end of surgery in order to control the wound healing events after the occurrence of the presumed tissue injuries. These solutions are supposed to act by increasing the lubrication and preventing the fibrin clots from adhering to other surfaces or by mechanically separating damaged tissues while they heal.

In WO 9112026 the prevention of unwanted surgical adhesion between two tissue surfaces is obtained by interposing, between the surfaces, phospholipid in suspension or solution in a surgically acceptable carrier, which can include hyaluronic acid. A viscous solution of a phospholipid, propylene glycol and water is also described. Another approach of precoating for the prevention of peritoneal adhesion formation has been to exogenously supply membrane components by the addition of phospholipids as shown in SE 45793.

Less viscous polymer solutions have been used as a tissue protective coatings during surgery in order to maintain the natural lubricity of tissues and organs and to protect the enclosing membrane. Precoating for tissue protection and adhesion prevention includes coating tissues at the beginning of surgery before a significant tissue manipulation and irritation can occur and continues throughout the operation so that a protective coating can be maintained on the tissues.

US 5,366,964 shows a surgical viscoelastic solution for promoting wound healing, which is used in direct contact with cells undergoing wound healing. The solution is intended for cell protection and cell coating during surgery and comprises one or several polymeric components. Hydroxypropylmethylcellulose and chondroitin sulphate are supposed to lubricate the tissue, while sodium hyaluronate would provide viscoelastic properties to the solution.

Several agents of today for treating postsurgical adhesions contain hyaluronic acid. For example US 5,409,904 describes solutions which reduce cell loss and tissue damage intended for protecting endothelial cells during ophthalmic surgery. The compositions used are composed of a viscoelastic material comprising hyaluronic acid, chondroitin sulphate, modified collagen, and/or modified cellulose. In WO 9010031 a composition is described for preventing tissue adhesion after surgery containing dextran and hyaluronic acid act which substances are supposed to act synergistically. In WO 9707833 a barrier material for preventing surgical adhesions is shown, which comprises benzyl esters or covalently crosslinked derivatives of hyaluronic acid.

A hyaluronic acid based agent manufactured by Pharmacia under the trademark Healon and originally intended as an intraocular instillation has been found to be the most effective agent up to now. However, hyaluronic acid is isolated from cock's crests and is thus very expensive as well as potentially allergenic even in small quantities and even more for large surfaces such as the peritoneum which has an area of about two m<sup>2</sup>.

The above mentioned hydrophilic polymeric solutions are mainly based on the viscosity of the high molecular weight polymer which often increases dramatically with increasing concentration. The polymer in question is often a polysaccharide as in US 4,994,277, in which a viscoelastic gel of biodegradable xanthan gum in a water solution for preventing adhesions between vital tissues is described. However, the major disadvantage of these polymers when used for reducing for example peritoneal adhesions as protective coatings during surgery or surface separation agents after surgery is that they do not significantly reduce adhesions because of their short residence

time in the peritoneal cavity. The result is that subsequent surgeries have to be performed on the patient.

Water-insoluble biocompatible compositions are shown in EP 0,705,878, which comprise a polyanionic polysaccharide combined with a hydrophobic bio-absorbable polymer.

EP 4,991,64 describes lubricating compositions comprising a hydrophilic polymer suspended in liquid carriers such as glycerol, propylene glycol, a fatty alcohol, a fatty acid, or polyethylene glycol.

WO 9319733 shows a moisturizer for epithelia which prevents abrasions and comprises bilayer particles of a hydrophobic adhesive polymer.

EP 713 708 shows a liquid polymer composition comprising a hydrophobic bioabsorbable polymer which will dissolve or disperse etc. in a hydrophilic liquid polymer. On contact with water the composition undergoes a phase separation resulting in the formation of an amorphous, liquid-crystalline or partially crystalline mass which is used in implants such as post surgical adhesion barriers.

WO 9508981 shows a contraceptive composition comprising a hydrophobically modified polysaccharide selected from cellulose, chitosans and mixtures thereof, which may be used as a personal care lubricant.

Until now no product has exhibited a pronounced reduction of the adhesion formations in clinical trials. Neither does any of the above-mentioned documents eliminate the problems of currently applied formulations for preventing postoperative adhesion between organs or parts of organs caused by tissue manipulation.

The purpose of the present invention is to provide a composition for lubricating and separating tissues and biological membranes from adjacent membranes or membranes from adjacent cells or tissues. A composition according to the invention provides an effective temporary replacement



of the normal protective layers by adhering to membrane surfaces by means of bioadhesion (mucoadhesion). An effective wound separation for preventing postsurgical adhesions between tissues is accomplished by the composition whereby the material of the composition provides a material which is not easily removed from its site of action. In order to achieve these purposes the method according to the invention has obtained the characterizing features of claim 1.

In order to further explain the invention reference is made to the accompanying drawings in which

FIG 1 is a diagram showing the difference in adhesion formation after surgery of mice between a control group and two polymers according to the invention as well as a comparative compound; and

FIG 2 is a diagram showing the number of adhesion attachments to different intraabdominal structures after surgery as in FIG 1.

A composition according to the invention contains a high molecular weight water soluble cationic polymer having hydrophobic groups which can attach to membranes. The hydrophobic groups are covalently bonded to the polymer and are designed to penetrate into membranes which on their surfaces are covered with a mesothelial, synovial, or mucus layer. The mesothelium covers serous membranes such as peritoneum, pericardium, and pleura, and synovial membranes cover surfaces of joints and tendon sheets. Compositions according to the invention are especially adapted to be used on a mesothelial/serosal membrane. However, they can also be used for lubricating and preventing adhesions in other organs and/or carrying different compounds such as drugs or vaccines to body cavities or other organs.

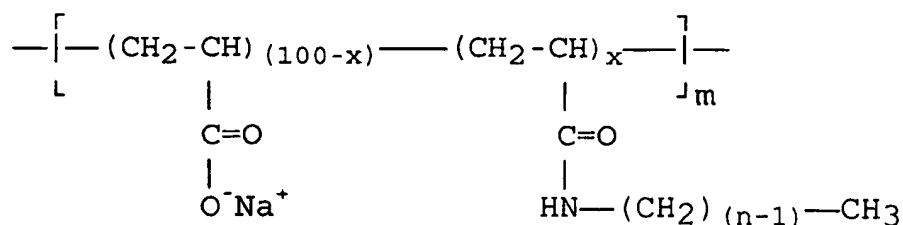
The cationic polymers according to the invention have a hydrophilic backbone to which a low number of hydrophobic side chains, or tails, are covalently bonded. The polymers to be hydrophobized can be synthetic as well as natural.

Among the synthetic polymers sodium polyacrylate and polyethylene glycol can be mentioned.

The most preferred polymers to be modified, however, are different kinds of polysaccharides such as cellulose, starch, chitin, chitosan, alginate, hyaluronic acid, and derivatives thereof. Of these cellulose is insoluble in water so it is made soluble in an aqueous solution by grafting the polysaccharide with for example ethylene oxide and ethyl groups. A substitution with more hydrophobic substituents, such as methyl and hydroxypropyl, can also render such polymers water soluble. Thus, suitable cellulose derivatives are ethyl-hydroxyethyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, methyl cellulose, and hydroxypropyl cellulose.

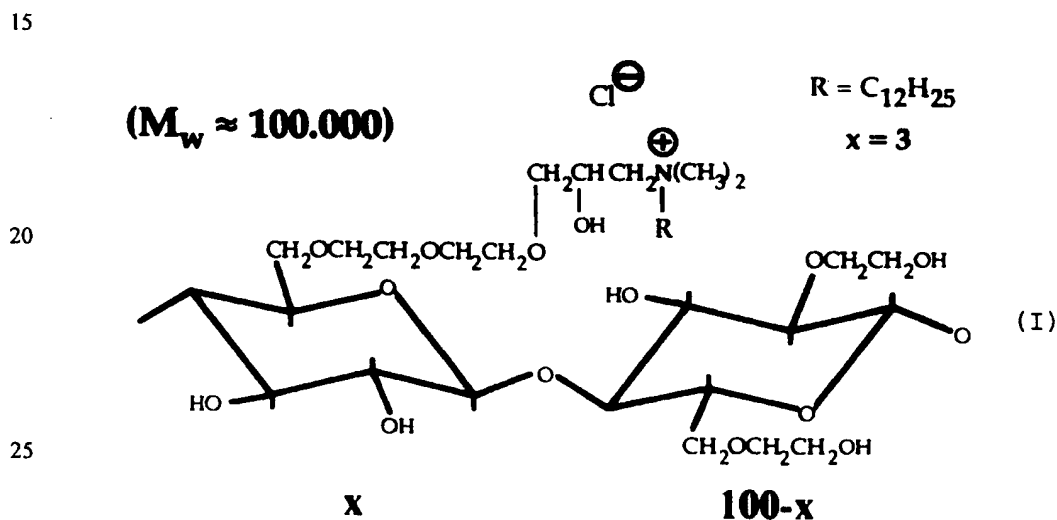
In such grafted hydrophobically modified water soluble polymers the number of hydrophobic groups is usually small. Preferably, 1-5 % of the monomer units are modified, i.e. below 5 mol% of hydrophobic side chains, or tails, are attached to the hydrophilic backbone. By combining a hydrophilic backbone with lipophilic pendant groups an affinity for the lipid/water interface is accomplished. Furthermore, solutions of hydrophobically modified polymers have been found to have higher viscosities than the solutions of their unmodified analogue. This feature of a polymer according to the invention assists in mechanically separating adjoining traumatized surfaces.

The hydrophobic modification can be performed with different kinds of substituents. Preferably, alkyl or alkylaryl groups are used. Most preferably, the substituents are alkyl chains with 10 to 18 carbon atoms. Compounds containing one or more benzene rings as well as cholesterol can also be used. An example of the hydrophobic modification of a synthetic polymer, i.e. sodium polyacrylate, is shown below.



In the hydrophobically modified form it is preferred that  $x=3$ ,  $n=12$ , and  $m=20$  in the cationic polymer. However, other combinations can also be used.

In compositions according to the invention cationic polymers are most effective in lubricating and separating membranes. An example of a water soluble cationic hydrophobically modified hydroxyethyl cellulose is shown below.



The preferred polysaccharides can be used separately or according to a further aspect of the invention be combined with certain lipids, such as polar lipids, to confer an enhanced hydrophobic effect to the polysaccharides. By increasing the hydrophobic bonding with such lipids the strength of the attachment between the polysaccharide and the peritoneal membrane is enhanced. This improved effect is especially obtained when the polar lipids are trans-

formed into a vesicular state which then can form an association complex with the actual polysaccharide.

When used in compositions such lipids must be able to form vesicle dispersions, and the vesicles formed should be able to associate with the polysaccharides into a colloidal complex. In order to avoid any risk of toxicological effects being obtained phospholipids and galactolipids are preferred since these lipids are naturally occurring components in cell membranes.

Polysaccharides containing positive charges can be combined with phospholipids having at least 10 % (w/w) of negatively charged species which by mechanical dispersion processes are able to form kinetically stable vesicles with the polysaccharide in question. Other polysaccharides can be combined with neutral or zwitterionic polar lipids which similarly can form kinetically stable vesicles by mechanical dispersions with the polysaccharide in question.

An additional advantage of the present invention is that the treatment of the infection and/or the inflammation obtained after surgery as well as the accompanying fibrin formation can be accomplished by a carrier of drugs being provided by means of what is called bioenhancing. In this embodiment of the invention the vesicles obtained can include different compounds and they can be used as carriers of these compounds to a specific site of action on a membrane in order to be released there. An example of such a compound can be a drug with the ability of preventing fibrin formation or enhancing fibrin dissolution, and there are several compounds available which inhibit the tissue plasminogen activator (tPA). Other suitable but not limiting compounds for site-specific delivery comprise drugs, vaccines, diagnostic agents etc. Preferably, a compound carried by the vesicles is an X-ray contrast agent, a drug enhancing fibrin degradation, e.g. tissue plasminogen activator (tPA), urokinase plasminogen activ-

ator (uPA), inhibitors of plasminogen activating inhibitors (PAI's), or a component that affect posttraumatic inflammations, e.g. agents that affect cytokines and growth factors, steroids and non-steroidal antiinflammatory drugs.

5        Further crosslinking of adjacent chains in the cationic polymers also increase the efficiency of a composition according to the invention. For example, inositolhexaphosphate (IP6), also known as phytic acid, has exhibited unexpected good effects when combined with  
10 cationic polymers according to the invention. These positive effects are considered to be due to adjacent chains of the cationic polymer being crosslinked with IP6 which results in an enhanced barrier effect. Furthermore, the favourable tissue response of this polymer-IP6  
15 combination takes advantage of the antioxidant functions and antiinflammatory effects of IP6.

      An effective concentration of a hydrophobized cationic polymer in a composition according to the invention ranges from about 0.2 to about 5 weight%, preferably from  
20 about 0.5 to about 2 weight%, of the final liquid composition. The composition comprises polymers formulated with a pharmaceutically acceptable carrier according to well known, conventional methods. Preferably, buffered aqueous media are used which contain pharmaceutically acceptable  
25 salts and buffers.

      A composition according to the invention can be added to the surgical area of the operation before and/or at the end of surgery. However, the compositions can for example also be added to the rinsing liquid for peritoneal  
30 dialysis in order to lubricate and prevent adhesions when used repeatedly.

      When a composition according to the invention is added to exposed vital tissues of body cavities such as the mesothelium, synovia or mucus covered cavities a thin film  
35 is formed on the membranes, and a steric stabilization is

achieved at the site of treatment. By the attachment of the cationic polymer to the membrane it is avoided that the film is removed by for example respiratory, cardiac, peristaltic, or other movements of the organs. It is important  
5 that the aqueous polymers as well as the lipids can be sterilized and that they are biologically acceptable. Preferably, the hydrophobized polymer material is a harmless biologically inert polymer which is not metabolized by the body, but a biodegradable material which dissolves over  
10 time in vital tissues can also be used.

The attachment of a polymer according to the invention to the membrane surface is accomplished by hydrophobic tails protruding from the polymer chain interact with the serous or mucous membrane. By this hydrophobic interaction  
15 a stabilizing effect is achieved in the membrane which facilitates its healing process. The polymers will thus remain in contact with the serous membranes for a considerably longer time than other polymers according to the state of the art.

20 By the use of a hydrophilic backbone with a low number, i.e. less than 5 mol%, of attached hydrophobic side chains, or tails, a structure is obtained which assists in keeping the membranes apart. The polymer forms an arch-like structure between the hydrophobic tails embedded in the  
25 biological membrane. In the space formed between these points the structure will be strengthened when the polymers are positively charged since they are attracted by the inherently negatively charged membranes. This attracting effect of opposite charged membranes has an additional  
30 advantage in increasing the lubricating and separating properties of the polymer by the friction between polymer and membrane being reduced.

Thus, a hydrophobic cationic polymer according to the invention can bind to a membrane by means of hydrophobic  
35 and/or electrostatic interactions.

**Examples.**

The present invention will now be explained more in detail with reference to the following examples.

5     **Example 1.**

A mixture of 20 g of phospholipids comprising 88 % (w/w) phosphatidylcholine, 8 % phosphatidylinositol, and 4 % phosphatidylglycerol was dissolved in ethanol and then freeze-dried in order to obtain molecular mixing. The  
10     purity of these commercial lipid samples (all from Sigma Chemical Co.) was 95 %. By using an Ultraturrax mixer the lipids were dispersed in 500 g of water which previously had been made isotonic by the addition of glycerol. When the dispersion became a semitransparent solution - which  
15     was stable when stored - a solution of 25 g of the polymer (I) in 500 g of an isotonic glycerol-water solution was added. The formulation was sterilized in glass ampoules.

**Example 2.**

A dispersion of 15 g of digalactosyl-diglycerides  
20     prepared from wheat gluten by chromatographic separation as described by Carlsson et al. (Cereal Chemistry 55 (1978) 168-179) in 500 g of physiological (isotonic) saline was prepared by means of ultrasonification. After the transformation of the dispersion from multi-bilayer liposomes to  
25     uni-bilayer vesicles, as evident from the semitransparent appearance and the lack of birefringence of the particles when viewed in the polarizing microscope, 500 g of a solution of 10 g of hydrophobically modified ethyl hydroxyethyl cellulose was added. The ultrasonification was  
30     allowed to continue for a few minutes in order to improve the polysaccharide hydrocarbon chain penetration into the vesicles. Samples were transferred to 10 g glass bottles and sterilized before use.

**Example 3.**

35     The formation of adhesions was quantified and the morphologic response in the tissue was studied after sur-

gery of NMRI mice as an animal model. The effects of two polymers according to the invention were compared with a phospholipid as a reference.

Compound I (1% wt) was obtained as LM-200 from  
5 Bionord AB, Gothenburg, Sweden. Compound II (1% wt) was a hydrophobically modified ethyl-hydroxyethyl cellulose obtained from Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden. As comparison sphingomyelin (95% wt) was used which was obtained from Swedish Dairy's Association, Research  
10 Department, Lund, Sweden. The sphingomyelin was diluted in sterile water to a final concentration of 1% wt. All compounds were kept refrigerated until used.

The animals - 142 female NMRI mice weighing 30-35 g - had free access to pellets and water before and after  
15 surgery. They were randomly allocated to different experimental groups.

One group (n=6) was sham-operated. Three groups were subjected to a short term treatment and three groups were given a prolonged treatment. The control groups consisted  
20 of animals which in a similar fashion received an equal volume of saline solution.

The short term treatment consisted of an intra-peritoneal injection (i.p.) of either compound I (n=21), compound II (n=21) or the comparative compound (n=16) after  
25 closure of the abdominal cavity. The controls received saline only (n=16). The volume load was 0.75 ml (0.25 mg/g body weight). The groups receiving prolonged treatment were given an i.p. injection of either compound I (n=21), compound II (n=21) or the comparative compound (n=16) not only  
30 at the time of the operation but also on day 2 and day 3 postoperatively. The controls received saline only (n=16). In all cases the volume load was the same (0.75 ml). When the morphological analysis of tissue response was performed two animals from each group were sacrificed at day 1, 3, 5  
35 and 7 for.



Surgery was performed after anesthesia induced and maintained by inhalation of methoxyflurane (Metofane<sup>®</sup>, Pitman Moore Inc., Mundelein, USA). Then the abdomen was shaved and opened with a 2.5 cm midline incision. The abdominal wall was held upwards with two forceps attached to *linea alba*, thereby exposing the interior. A 2 x 15 mm defect in the parietal peritoneum was created on each side of the lateral side wall by a sharp incision along a metal ruler followed by an excision with a pair of scissors. The lesion was closed with four 5/0 silk sutures, one at each end of the lesion and the other two at an equal distance between them. The midline incision was closed in two layers by a continuous 3/0 polyglycolic mass closure (Dexon<sup>®</sup>, Davies and Geck, USA) according to well-known procedures.

At day 1, 3 and 5 post-operatively, two animals were sacrificed from each group by the administration of an overdose of isoflurane (Forene<sup>®</sup>, Abbott Laboratories Ltd., Queensborough, England) and biopsies were taken for morphological studies. Seven days after the operation the remaining animals were sacrificed. The abdominal cavity was widely opened with a U-shaped incision with its base to the right of the animal. The flap was then turned over to the right for displaying the parietal peritoneum of the anterior abdominal wall and the adhesions attached to it. The lesions previously induced to the peritoneum were identified by the suture lines. The evaluation was performed by measuring with a calliper the length of the original lesion and that of the individual adhesion attachments. The extent of the peritoneal defect covered with adhesions could then be calculated according to the following formula as previously described:

$$\text{Adhesions (\%)} = \frac{\text{Sum of attachments (mm)}}{\text{Excision site (mm)} \times 100}$$

In addition, the location and the number of attachments were noted.

The injured area including the parietal peritoneum, the muscular layer and the skin was excised in order to  
5 study the morphology. The specimen was fixed in Bouin's solution for 24 hours, dehydrated and embedded in paraffin wax. Sections having a thickness of 5  $\mu$ m were cut out and stained with haematoxylin and eosin.

In order to determine the significance of differences  
10 in adhesion formation between groups the values obtained and given as mean (SEM) were statistically evaluated by means of the non-parametric Mann-Whitney U test. A p value of less than 0.05 was considered to be statistically significant.

15 None of the animals had obtained any adhesions at the beginning of the operation. All animals developed adhesions, except for two animals which had received compound I as a prolonged treatment. No bowel obstruction or displayed signs of a generalized malaise had developed in any animal.

20 No adhesion formation was observed postoperatively in the sham-operated group except for the midline incision. As shown in FIG 1 there was no significant difference in adhesion formation between the control group [69.0 (4.5) %] and the group having obtained the comparative compound  
25 [69.7 (6.7) %, p=0.733] as a short term treatment. The group treated with compound II exhibited a significantly lesser adhesion formation [48.1 (5.1) %, p=0.012] than the control, as did the group treated with compound I [44.2 (6.3) %, p=0.013]. A repeated injection of the comparative  
30 compound two and three days postoperatively resulted in a reduction in adhesion formation [72.4 (4.5) %, p=0.049] compared with saline. Significantly less adhesions were seen in the groups receiving the long term treatment with compound II [47.6 (6.5) %, p=0.003] or compound I [24.4  
35 (5.3) %, p<0.001] compared with the control group given

three injections of NaCl [84.5 (6.2) %]. No difference in effect was obtained between the group receiving compound II as a short term treatment [48.1 (5.1) %] compared with the group receiving compound II as a prolonged treatment [47.6 (6.5) %,  $p=0.747$ ]. The adhesion reducing effect of compound I was significant with the short as well as the long term treatment. However, in contrast to compound II compound I was significantly more effective when given as a prolonged treatment [24.4 (5.3) %] compared with the short term treatment [44.2 (6.3) %,  $p=0.032$ ]. The results are shown in FIG 1.

The number of adhesion attachments to different intraabdominal structures was also determined (FIG 2). The structures studied were omentum/pelvic fat bodies (PFB), bowel (B), or other (O, predominantly liver). As shown in FIG 2 there was no significant effect of compound I compared with the control on the number of adhesions when given as a short term treatment (PFB,  $p=0.098$ ; B,  $p=0.114$ ; O,  $p=0.717$ ). The same result was obtained with compound II (PFB,  $p=0.205$ ; B,  $p=0.103$ ; O,  $p=0.717$ ). The comparative compound sphingomyelin reduced the number of attachments to omental structures but not to the bowel or to other parts of the abdominal cavity (PFB,  $p=0.049$ ; B,  $p=0.434$ ; O,  $p=0.342$ ).

When compound I was given in three consecutive doses a significant reduction of the number of attachments to both the omentum ( $p=0.023$ ) and the bowel ( $p<0.001$ ) was obtained, but not to other structures ( $p=0.147$ ). Compound II reduced the number of attachments to the bowel and other structures (B,  $p=0.004$ ; O,  $p=0.032$ ) but not to omentum/pelvic fat bodies ( $p=0.596$ ). When given as a long term treatment the comparative compound exhibited no reducing effect at all on the number of attachments to any of the structures involved (PFB,  $p=0.720$ ; B,  $p=0.091$ ; O,  $p=0.888$ ). When compound I was given as a prolonged

treatment and compared with that group which had received the short term treatment significantly fewer adhesions were obtained to both omentum/pelvic fat bodies and bowel ( $p=0.023$ ;  $p=0.032$ ) but not to other structures ( $p=0.549$ ).

5 The data obtained are summarized in FIG 2.

When the tissue response was studied an inflammatory reaction could be seen at the site of adhesion formation. This was observed not only at the attachment site and in the abdominal wall but also in the adherent non-injured  
10 tissue. On day 1 post-surgery polymorphonuclear neutrophils dominated and later macrophages were abundant. A uniform morphologic appearance was seen on specimens obtained from all groups, and there was no difference between the treatment groups and the control group. Furthermore, no difference  
15 was obtained between the groups given short term treatment and long term treatment.

## CLAIMS

1. A composition for lubricating and separating tissues and biological membranes from adjacent membranes or membranes from adjacent cells or tissues comprising a hydrophobized polymer, characterized in that said hydrophobized polymer is a biologically acceptable water soluble cationic polymer with hydrophobic groups covalently bonded thereto and binds to a membrane, said membrane on its surface having a mesothelial, synovial, or mucus layer.
2. A composition as claimed in claim 1, characterized in that said water soluble cationic polymer is based on a synthetic polymer.
3. A composition as claimed in claim 2, characterized in that said synthetic polymer is sodium polyacrylate or polyethylene glycol.
4. A composition as claimed in claim 1, characterized in that said water soluble cationic polymer is based on a natural polymer.
5. A composition as claimed in claim 4, characterized in that said natural polymer is a polysaccharide.
6. A composition as claimed in claim 5, characterized in that said polysaccharide is selected from the group comprising cellulose, starch, chitin, chitosan, alginate, hyaluronic acid, and derivatives thereof.
7. A composition as claimed in claim 6, characterized in that said cellulose derivative is selected from the group comprising ethyl-hydroxyethyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, methyl cellulose, and hydroxypropyl cellulose.

8. A composition as claimed in claim 1, c h a r a c -  
t e r i z e d in that said hydrophobic groups are  
selected from the group comprising alkyl groups, alkylaryl  
groups, compounds containing one or more benzene rings, and  
5 cholesterol.
9. A composition as claimed in claim 8, c h a r a c -  
t e r i z e d in that said alkyl groups have 10 to 18  
carbon atoms.
10. A composition as claimed in claim 8 or 9, c h a -  
r a c t e r i z e d in that said hydrophobic groups  
comprise 1-5 % of the monomer units in said water soluble  
cationic polymer.
11. A composition as claimed in any of claims 1 to 10,  
c h a r a c t e r i z e d in that said water soluble  
15 cationic polymer is combined with inositolhexaphosphate in  
order to improve the barrier effect.
12. A composition as claimed in claim 6, c h a r a c -  
t e r i z e d in that said hydrophobic group covalently  
bonded to said polysaccharide is combined with a lipid in  
20 order to improve the bonding to the membrane.
13. A composition as claimed in claim 12, c h a r a c -  
t e r i z e d in that said lipid can form a vesicle  
dispersion which associate with said polysaccharide into a  
colloidal complex.
- 25 14. A composition as claimed in claim 12 or 13, c h a -  
r a c t e r i z e d in that said lipid is a phospholipid  
or a galactolipid.
15. A composition as claimed in claims 11 to 14, c h a -  
r a c t e r i z e d in that said cationic polymer is  
30 combined with a phospholipid having at least 10 % (w/w) of  
negatively charged species.

16. A composition as claimed in claims 12 to 14, c h a -  
r a c t e r i z e d in that said polysaccharide is  
combined with a neutral or a zwitterionic polar lipid to  
kinetically stable vesicles.

- 5 17. Use of a composition as claimed in any of claims 13-16  
for the delivery of drugs, vaccines, diagnostic agents etc  
to a specific site of action on a membrane in order to be  
released there.

**AMENDED CLAIMS**

[received by the International Bureau on 12 December 1998 (12.12.98);  
original claims 1-17 replaced by amended claims 1-16 (3 pages)]

**CLAIMS**

1. A composition for lubricating and separating tissues and biological membranes from adjacent membranes or membranes from adjacent cells or tissues comprising a hydrophobized polymer, c h a r a c t e r i z e d in that said  
5 hydrophobized polymer is a biologically acceptable water soluble cationic polymer with hydrophobic groups covalently bonded thereto and binds to a membrane, said membrane on its surface having a mesothelial, synovial, or mucus layer.
- 10 2. A composition as claimed in claim 1, c h a r a c t e r i z e d in that said water soluble cationic polymer is based on a synthetic polymer.
3. A composition as claimed in claim 1, c h a r a c t e r i z e d in that said water soluble cationic polymer  
15 is based on a natural polymer.
4. A composition as claimed in claim 3, c h a r a c t e r i z e d in that said natural polymer is a polysaccharide.
5. A composition as claimed in claim 4, c h a r a c t e r i z e d in that said polysaccharide is selected  
20 from the group comprising cellulose, starch, chitin, chitosan, and derivatives thereof.
6. A composition as claimed in claim 5, c h a r a c t e r i z e d in that said cellulose derivative is  
25 selected from the group comprising ethyl-hydroxyethyl cellulose, carboxymethyl cellulose, hydroxyethyl cellulose, methyl cellulose, and hydroxypropyl cellulose.
7. A composition as claimed in claim 1, c h a r a c t e r i z e d in that said hydrophobic groups are  
30 selected from the group comprising alkyl groups, alkylaryl groups, compounds containing one or more benzene rings, and cholesterol.



8. A composition as claimed in claim 7, c h a r a c -  
t e r i z e d in that said alkyl groups have 10 to 18  
carbon atoms.
9. A composition as claimed in claim 7 or 8, c h a -  
5 r a c t e r i z e d in that said hydrophobic groups  
comprise 1-5 % of the monomer units in said water soluble  
cationic polymer.
10. A composition as claimed in any of claims 1 to 9,  
c h a r a c t e r i z e d in that said water soluble  
10 cationic polymer is combined with inositolhexaphosphate in  
order to improve the barrier effect.
11. A composition as claimed in claim 4, c h a r a c -  
t e r i z e d in that said hydrophobic group covalently  
bonded to said polysaccharide is combined with a lipid in  
15 order to improve the bonding to the membrane.
12. A composition as claimed in claim 11, c h a r a c -  
t e r i z e d in that said lipid can form a vesicle  
dispersion which associate with said polysaccharide into a  
colloidal complex.
- 20 13. A composition as claimed in claim 11 or 12, c h a -  
r a c t e r i z e d in that said lipid is a phospholipid  
or a galactolipid.
14. A composition as claimed in claims 11 to 13, c h a -  
r a c t e r i z e d in that said cationic polymer is  
25 combined with a phospholipid having at least 10 % (w/w) of  
negatively charged species.
15. A composition as claimed in claims 12 to 14, c h a -  
r a c t e r i z e d in that said polysaccharide is  
combined with a neutral or a zwitterionic polar lipid to  
30 kinetically stable vesicles.

16. A composition as claimed in claims 12 to 15, c h a -  
r a c t e r i z e d in that said vesicle dispersion  
contains drugs, vaccines, diagnostic agents etc to be  
delivered at a specific site of action on a membrane in  
5 order to be released there.

FIG 1

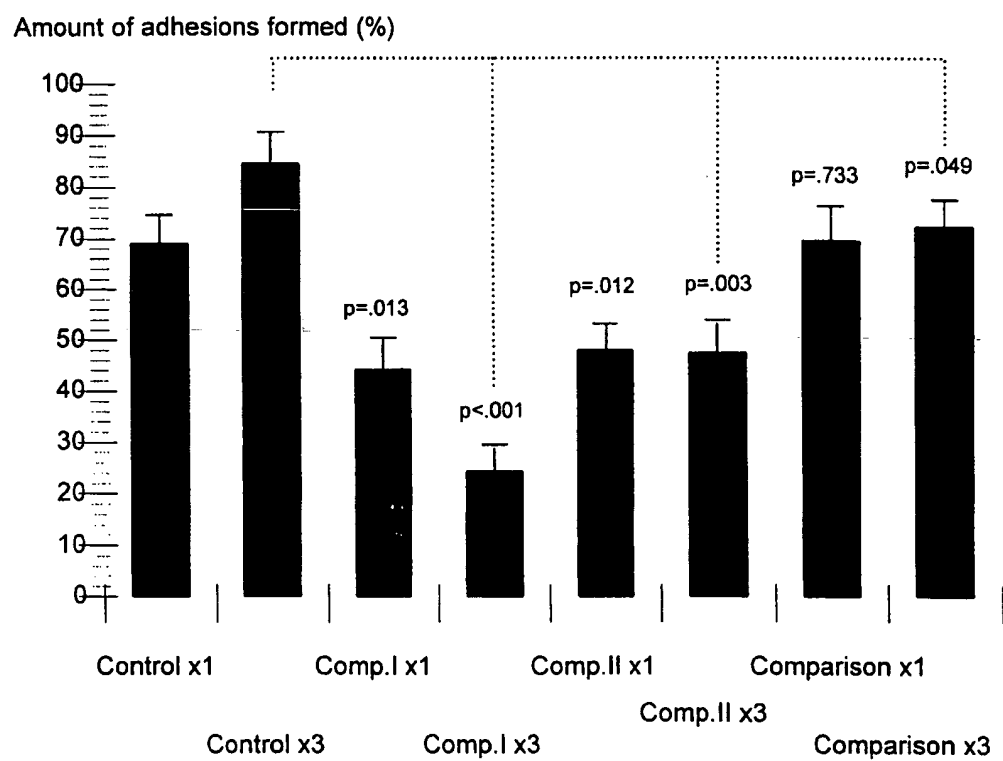
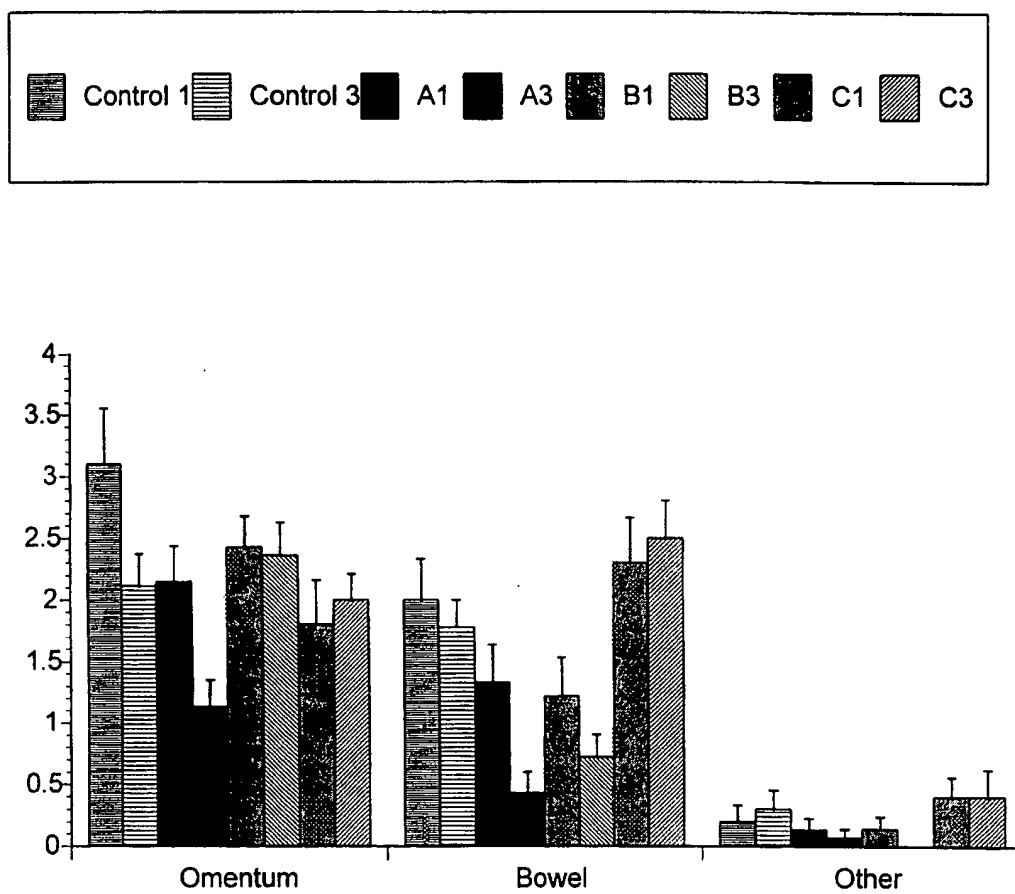


FIG 2



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01371

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/725, A61K 31/715, A61K 9/127

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, WPI

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9112026 A1 (MACNAUGHT PTY LIMITED), 22 August 1991 (22.08.91)	1-10,12-15
A	--	11,16-17
X	WO 9707833 A2 (FIDIA ADVANCED BIOPOLYMERS, S.R.L.), 6 March 1997 (06.03.97)	1,4-10
A	--	2-3,11-17
X	EP 0499164 A1 (C.R. BARD, INC.), 19 August 1992 (19.08.92)	1,4-10
A	--	2-3,11-17

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

20 October 1998

Date of mailing of the international search report

01 -11- 1998

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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01371

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9010031 A1 (PHARMACIA AB), 7 Sept 1990 (07.09.90)  --	1-17
A	WO 9632929 A1 (ALCON LABORATORIES, INC.), 24 October 1996 (24.10.96)  -- -----	1-17

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01371

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17  
because they relate to subject matter not required to be searched by this Authority, namely:  
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

27/07/98

International application No.

PCT/SE 98/01371

Patent document cited in search report			Publication date	Patent family member(s)		Publication date
WO	9112026	A1	22/08/91	NONE		
WO	9707833	A2	06/03/97	AU	6930096 A	19/03/97
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				SE	8900586 A	22/08/90
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				US	5409904 A	25/04/95
				US	5578578 A	26/11/96